

EXHIBIT 7



US006984391B2

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Fox et al.

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(54) **COMPOSITIONS AND METHODS FOR
 DELIVERY OF SKIN COSMECEUTICALS**

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 424/78.03

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 514/844, 846

See application file for complete search history.

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(57) **ABSTRACT**

New cosmetic compositions are disclosed that are effective
 both as moisturizers and skin sloughing agents. The com-
 positions contain neutralized weak organic acids that when
 applied over time to the skin in appropriate formulations will
 gradually increase in acidity to pH 5.5 or less, for example
 about pH 4.5, without causing skin irritation while exhibit-
 ing increasing activity in skin renewal effects.

29 Claims, 1 Drawing Sheet

U.S. Patent

Jan. 10, 2006

US 6,984,391 B2

Fig. 1

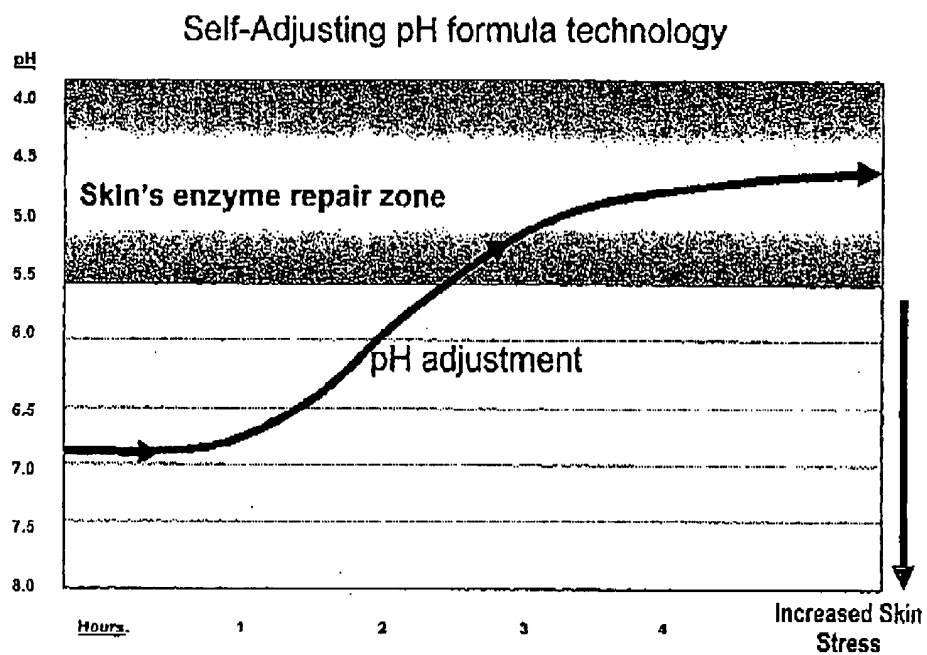
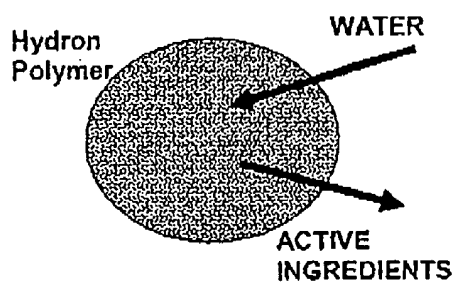


Fig. 2



US 6,984,391 B2

1

COMPOSITIONS AND METHODS FOR DELIVERY OF SKIN COSMECEUTICALS

This application claims the benefit of Provisional Application No. 60/357,466, filed Feb. 15, 2002.

BACKGROUND ART

1. Field of the Invention

The invention concerns compositions and methods for formulating and delivering cosmeceutical skin preparations. The disclosed compositions are particularly adapted for delivery of acidic agents for skin renewal and treatment agents.

2. Description of Related Art

Human skin acts as a protective barrier to both physical and chemical insults. The properties of the cutaneous layer are important to the cosmetic industry in developing effective and safe skin preparations in addition to medical concerns in treating damage to the skin surface in conditions such as acne.

The epidermal surface is acidic and has been the subject of studies on epidermal permeability and formation. The chemistry and function of dry skin and moisturizers has been extensively reviewed (Loden and Howard, eds, 2000) in the context of understanding the factors involved in developing skin moisturizers and protective formulations.

Skin homeostasis normally is maintained at about pH 5 allowing lipid barrier repairs. At more neutral or alkaline pH, skin repair is inhibited (Mauro, 1998). Studies have shown that there are racial differences in the stratum corneum pH gradient at least with respect to the surface layers. Significant differences between Caucasian and black African-American skin was reported by Berasdesca, et al. (1998), although no differences were found in the deeper stratum corneum layers.

Mauro et al. states that the skin's lipid barrier is impeded at neutral pH, independent of ionic effects. Epidermal permeability barrier homeostasis requires the post-secretory processing of polar lipid precursors into nonpolar lipid products within the stratum corneum (SC) interstices by a family of lipid hydrolase enzymes. A specific requirement for beta-glucocerebrosidase (beta-GlcCer'ase), which exhibits a distinct optimum acidic pH, is particularly well documented. The investigators sought to determine whether the recovery of the barrier after acute insults requires acidification of the SC. They examined permeability barrier recovery by assessing changes in transepidermal water loss (TEWL), SC membrane ultrastructure utilizing ruthenium tetroxide (RuO₄) postfixation, and beta-GlcCer'ase activity by *in situ* zymography at an acidic vs. neutral pH. Barrier recovery proceeded normally when acetone-treated skin was exposed to solutions buffered to an acidic pH. In contrast, the initiation of barrier recovery was slowed when treated skin was exposed to neutral or alkaline pH, regardless of buffer composition. In addition, enhancement of the alkaline buffer-induced delay in barrier recovery occurred with Ca²⁺ and K⁺ inclusion in the buffer. Moreover, the pH-dependent alteration in barrier recovery appeared to occur through a mechanism that was independent of Ca²⁺ or K⁺ controlled lamellar body secretion, since both the formation and secretion of lamellar bodies proceeded comparably at pH 5.5 and pH 7.4. Exposure to pH 7.4 (but not pH 5.5) resulted in both the persistence of immature, extracellular lamellar membrane structures, and a marked decrease in the *in situ* activity of beta-GlcCer'ase. These results suggest first that an acidic extracellular pH is necessary for the initiation of barrier

2

recovery, and second that the delay in barrier recovery is a consequence of inhibition of post-secretory lipid processing.

Kligman (2000) states "... our concept of humectants (as moisturizers) falls short of explaining how they work. It is worth repeating that used alone they are not much good. It is only when they are properly formulated with other ingredients that their potential benefits are realized. Other factors such as pH also have to be taken into account, because proteases which lead to orderly desquamation of horny cells within the stratum corneum are activated only at acid pHs of 4 to 5. Also the various hydrolytic enzymes, which are found in the stratum corneum and which are essential to the formation of the intercorneocyte lipids that establish the impermeability of the barrier, are activated only at acid pHs" (Kligman, 2000).

Feingold and Elias (2000) state that the epidermal surface has been known for many years to be acidic, but the role of this acidic pH of the stratum corneum in barrier homeostasis was unknown. It is well recognized that beta-glucocerebrosidase is most active at pH 5.5. Recently, we have examined barrier recovery of an acidic vs. neutral pH. Barrier recovery proceeded normally when acetone treated skin was exposed to solutions buffered to an acidic pH. In contrast, barrier recovery was delayed when treated skin was exposed to neutral or alkaline pH regardless of buffer composition" (in *Dermatology*, eds. Loden and Maibach, 2000). These results indicate that an acidic extracellular pH in the SC is required for normal extracellular lipid processing and normal barrier homeostasis.

The majority of marketed skin treatment preparations use emulsifying agents that are non-volatile and accordingly remain on the skin until removed by cleansing. Most facial care cosmetics are formulated with about 5-7% emulsifying agents, at a pH of 6.5-8.0 to insure product stability, and contain on average about 75% water. After application to the skin the water used in the formulation evaporates off the skin quickly, leaving up to approximately a 20% concentration of emulsifying agents on the skin. This high level of emulsifying agent is capable of emulsifying the natural lipids in the skin which can be removed on cleansing the skin. The net result is detrimental to the skin for two reasons: the residual pH of 6.5 to 8.0 is not favorable for the repair of the skin's lipid barrier layer and the residual high level of emulsifying agents is conducive to removal of the natural lipids in the skin leading to an even drier skin condition. The stress of the emulsifying agent residue causes overstimulation of the skin, increased oil production, irritation and blemishes. Stronger emulsions are difficult to neutralize for older skin and sensitive skin. Harsh emulsifiers may actually damage skin by dissolving the skin's protective lipid barrier, which is essential to healthy looking skin. Additionally, perspiration may remove the active ingredients deposited on the skin.

DEFICIENCIES IN THE PRIOR ART

The inventors have observed that an acidic skin care agent is desirable for the reasons given above, but many acidic agents that have been used in skin products have a pH low enough to cause irritation or slight burning. The irritation and burning can be cumulative over repeated application. The acids are effective at low pH, however, they are not available in formulations for periods of time of at least several hours at optimal effective pHs. A stable shelf-life is necessary for skin care formulations, which generally requires a pH of no lower than 6.0. A pH of 6.0 or more

US 6,984,391 B2

3

insures that the product is stable, but is too high to promote effective rebuilding of the skin's protective barrier.

SUMMARY OF THE INVENTION

The invention recognizes the need to provide an environment of below pH 5.5, and preferably between about pH 4.5 and 5.0 to promote activity of the skin enzymes involved in repairing a damaged lipid skin barrier.

The invention addresses the problem of providing an ideal pH for cosmeceuticals and skin treatments. This is accomplished by providing an ammonium salt of a selected weak organic acid to the skin in a formulation that allows slow evaporation after skin contact, resulting in release of ammonia and a gradual decrease in pH at or below 5.5. The decrease in pH may take place over several hours. Active ingredient delivery of pharmaceutical ingredients and/or skin sloughing is enhanced as the pH becomes more acidic, but there is no irritation because the decrease in pH is gradual. At some point the pH of the parent acid is reached, adding a measure of control over the final pH.

As used herein, the disclosed preparations and formulations are termed cosmeceuticals, a hybrid term incorporating the concept of improving skin appearance by application of active ingredients that often serve a therapeutic role. Certain of the particular formulations disclosed have application in treatment of skin conditions with over the counter (OTC) drugs, such as the use of salicylic acid for acne treatment. The disclosed formulations provide, among other benefits, a method of controlling pH of salicylic acid on the skin to avoid irritation and burning and by causing a gradual lowering of pH from the neutral value at the initial application.

The disclosed novel product delivery system has many advantages over conventional products in regard to safety, efficacy and longevity of action.

There are many weak organic acids that are beneficial to the skin, such as salicylic acid, glycolic acid, lactic acid, malic acid, citric acid, tartaric acid and the like but which have a very low pH and can be irritating. Salicylic acid is a beta hydroxy acid and is an approved drug at 2% for the treatment of acne. Other acids include alpha and mixed alpha and beta-hydroxy acids that are used to slough off the top dead layers of the skin to improve skin appearance.

Selected acids are formulated into vehicles in the form of solutions, lotions, and/or creams that are well recognized for use in the preparation of skin products. The final products are adjusted to a pH value of 6.5-6.9 with an aqueous ammonia solution. This results in the formation of the ammonium salt(s) so neutralized. It is well known that certain salts of alpha hydroxy acids such as the sodium, potassium, ammonium, diethanolamine, triethanolamine etc. salts are humectants and when applied to the skin they are safe and non-irritating and are powerful skin moisturizers; but because the pH is at neutrality, these salts remain active only as humectants and will not enhance skin sloughing of scaly dry skin or enhance skin turnover. However, when the salts applied are the ammonium salts, as in the present invention, after application to the skin the ammonia gradually evaporates from the product, the pH drops and the moisturizer turns into an active acid for enhancing skin sloughing and turnover and in the case of salicylic acid becomes an active agent for the treatment of acne. The advantage of this system is that the pH drop occurs over time, typically three to four hours and so the product is never irritating to the skin and the activity increases with time.

4

It is also possible to build into these systems salts of skin protective polymers that are water soluble at pH 6 to pH 7 but which become insoluble at pH values below pH 5. Thus ammonium carboxymethyl cellulose, ammonium alginate, ammonium cartagheenate, ammonium polyacrylate, ammonium VA/Acrylate and the like are water soluble but after application to the skin the ammonia evaporates and a thin layer of water insoluble polymer is left on the skin which acts as a barrier to protect the skin, enhances skin moisturization and, because it is water insoluble, holds other important ingredients in contact with the skin.

There is one further major advantage that accrues in using the ammoniated system. It has been shown that the skin enzymes involved in repairing a damaged lipid skin barrier are only active at a pH of below 5.5. Almost every skin care preparation on the market today is at a pH of 6.5-7.5 and remains at this pH for as long as it remains on the skin thus interfering with the enzymes required for rebuilding the protective lipid barrier. In the disclosed compositions, the ammonia evaporates and the pH reaches the optimum level for enzymatic activity and skin barrier repair.

In certain embodiments, a self-adjusting emulsifier may be employed. As the emulsifier in the formulation evaporates, the composition self-adjusts to a lower pH which will typically be in the range of 3.5-5.5 matching the stratum corneum pH required for baling and enzyme production to repair the lipid barrier.

There are two types of emulsions: oil in water and water in oil. Formulations can be created to deliver 100% of the therapeutic ingredients on application to the skin and hold the ingredients when a polymer complex is used. Polymer based moisturizers create a protective barrier on the skin that captures moisture normally lost by the skin but at the same time allow the skin to readily exchange oxygen and carbon dioxide, unlike oil based moisturizers. The moisture cushion allows water to soften the skin.

The basic compositions may include numerous other ingredients, depending on the requirements of a particular skin type. Vitamins such as vitamin A palmitate, pro-vitamin B-5, vitamin E, vitamin D3 and vitamin C are illustrative of beneficial vitamins that may be added. Herbal and botanicals may also be included with the ammonium salts, including green tea, aloe vera, ivy extract, chamomile, watercress, silk, sea kelp, meadowsweet, ginkgo biloba, spirulina maxima, passion flower, witch hazel, pea extract, algae extract, apple, sugar cane, avocado oil, jojoba oil and evening primrose oil.

Delivery may be formulated in liposomes, ceramide III, triclosan, avobenzone, oxybenzone or in other numerous ways accepted in the art for formulating cosmeceuticals.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the natural pH repair zone for skin.

FIG. 2 schematically illustrates the moisture uptake by a polymer layer at the skin surface and concomitant release of active ingredients.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention relates to cosmetic moisturizing preparations that adjust from higher to lower pH after application. These formulations typically provide a pH level of about 4.5 by incorporating a fatty acid salt as a major ingredient. The salt is converted at least in part to a fatty acid which lowers the pH at the skin surface. This is illustrated

US 6,984,391 B2

5

in Table 1 where pH changes for selected moisturizing creams from different brands are compared.

Most moisturizers are formulated and packaged for long shelf life. Initially the pH is adjusted to about 6.0–7.5 to provide the stability required. This is accomplished by providing an ammonium salt of a selected weak organic acid to the skin in a formulation that allows slow evaporation after skin contact, resulting in release of ammonia and a gradual decrease in pH at or below 5.5. The decrease in pH may take place over several hours. Active ingredient delivery of pharmaceutical ingredients and/or skin sloughing is enhanced as the pH becomes more acidic, but there is no irritation because the decrease in pH is gradual. At some point the pH of the parent acid is reached, adding a measure of control over the final pH.

EXAMPLES

Example 1

Skin Moisturizing and Sloughing Preparation

Percent (w/w)	
<u>Part 1</u>	
Stearic Acid	2.55
Cetyl Alcohol	1.70
Glyceryl Stearate	1.25
Isopropyl Stearate	0.42
Caprylic/Capric Triglyceride	0.25
Lenolin	0.42
<u>Part 2</u>	
Deionized Water	qs 100.00
Glycolic Acid (70%)*	7.00
Ammonium Hydroxide (28%)	qs to pH 6.7
<u>Part 3</u>	
Propylene Glycol	7.50
SD Alcohol 40-2	5.00
Polyhydroxyethylmethacrylate	0.50
Steareth-20	0.02
<u>Part 4</u>	
Germaben II (ISP)	1.00
<u>Part 5</u>	
Fragrance	0.10

Example 2

Skin Facial Moisturizing and Sloughing Preparation

Percent (w/w)	
<u>Part 1</u>	
Octyl Methoxy Cinnamate	7.00
Benzophenone-3	3.00
Avobenzone	2.00
Stearic Acid	2.30
Cetyl Alcohol	1.50
Glyceryl Stearate	1.50
Dimethicone	1.00
Tocopheryl Acetate Cosmetic Grade	0.50
C ₁₂₋₁₅ Alkyl Benzoate	0.35
Stearyltrimethylsilane	0.25

6

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Percent (w/w)	
<u>Part 2</u>	
Vitamin A Palmitate	0.10
Terphenylacyl Ascorbate	0.10
<u>Part 3</u>	
Deionized Water	qs 100.00
Lactic Acid (88%)*	5.00
Disodium Edetate	0.20
<u>Part 4</u>	
Deionized Water	9.80
Propylene Glycol	7.50
SD Alcohol 40-2	5.00
Polyhydroxyethylmethacrylate	0.50
Aloe Vera Gel (200X)	0.10
Steareth-20	0.02
<u>Part 5</u>	
Ammonia Solution Strong (27%) (26° Baume)	qs to pH 6.7–6.9

Example 3

Acne Moisturizing and Treatment Cream

<u>Part 1</u>	
Caprylic/Capric Triglyceride	5.00
Glyceryl Stearate	3.00
Salicylic Acid*	2.00
Cetyl Alcohol	1.50
Stearic Acid	1.30
Beeswax	1.00
Cholesterol	0.25
BHT	0.05
<u>Part 2</u>	
Deionized Water	47.17
Propylene Glycol	5.00
Sodium Hyaluronate	3.00
Sodium PCA	2.00
Saccharide Isomerate	1.00
Disodium Edetate	0.20
<u>Part 3</u>	
Deionized Water	9.80
Propylene Glycol	7.50
SD Alcohol 40-2	5.00
Polyhydroxyethylmethacrylate	0.50
Aloe Vera Gel (200X)	0.10
Steareth-20	0.02
<u>Part 4</u>	
Ammonia Solution Strong (27%) (26° Baume)	qs to pH 6.7–6.9

*While the examples shown above utilize glycolic, lactic and salicylic acids, this does not preclude the use of other hydroxy or non-hydroxy acids having a moisturizing, skin sloughing or skin treatment effect.

Ammonium stearate was used as the emulsifying agent. Glycolic acid was neutralized with ammonia to form the ammonium salt. The salt was then formulated into a pharmaceutically acceptable cream selected for non-irritation to the skin and typically used in preparing cosmetic formulations. An all over moisturizer was prepared (Example 1), with similar preparations prepared for the face (Example 2), as an acne treatment cream (Example 3), for the fragile eye area (Example 4) and as a night cream (Example 5). The composition of Examples 1–3 is given above. For Examples 4 and 5, the different preparations used different concentrations of inactive ingredients that are well known in the art for producing moisturizing creams. Upon application to the

US 6,984,391 B2

7

skin, the ammonia gradually evaporated to form glycolic acid. The acid began sloughing of dead cells with greater activity over a period of 3-4 hours as the pH of the composition gradually decreased over this period to about 4.5.

Table 1 illustrates the change in pH over time of several cosmetic products by comparison to the disclosed product. It can be seen that the pH of Examples 1-4 decreases to 4.5 and remains at that level for several hours in contrast with comparable products whose pH shows little change from neutral.

TABLE 1

Product	pH*				
	Initial	1 Hour	2 Hours	3 Hours	8 Hours
Vaseline** Intensive Care Advanced Healing Lotion (Lot 04219PP10)	7.5	7.5	7.5	7.5	7.5
Vaseline** Intensive Care Dry Skin Lotion (Lot 679991)	7.0	7.0	7.0	7.0	7.0
Lubriderm** Skin Therapy (Lot 679991)	7.5	7.5	7.5	7.5	7.5
Neutrogena** Moisture for Sensitive Skin (Lot 1L9)	7.5	7.5	7.5	7.5	7.5
Ponds** Nourishleg Moisturizer Lotion (Lot 01190H02)	6.5	6.5	6.5	6.5	6.5
Neutrogena** Intensified Day Moisture (Lot 1M9)	7.0	7.0	7.0	7.0	7.0
L'Oréal** Active Daily Moisture Lotion (Lot PV108)	6.0	6.0	6.0	6.0	6.0
Nivea** Visage (Lot 99169)	7.0	6.5	6.5	6.5	6.5
Night of Olay** Lot 9160H	7.0	6.5	6.5	6.5	6.5
Nivea** Q-10 Cream (Lot 52840851)	7.0	7.0	7.0	7.0	7.0
Olay** Activating Hydration Lotion (Lot 903911)	7.0	7.0	7.0	7.0	7.0
Estée Lauder** Resilience (Lot CB9)	6.0	6.0	6.0	6.0	6.0
Clinique** Dramatically Moisturizing Lotion (Lot 037)	7.5	7.5	7.5	7.5	7.5
Lancôme** Reasrge Antiwrinkle Cream (Lot 0257)	6.0	6.0	6.0	6.0	6.0
Example 1	7.0	5.5	5.0	4.5	4.5
Example 2	7.0	5.8	5.0	4.5	4.5
Example 3	7.0	5.5	5.0	4.5	4.5
Example 4	7.0	5.5	5.0	4.5	4.5
Example 5	7.0	5.5	5.0	4.5	4.5

*ColorHast pH 0-14 pH paper, EM Science, Gibbstown, NJ 08027 was used for this test

**Registered Trademarks

The examples of the present invention were tested in 8 and 12 hour clinical studies, which proved extended performance as well as a reduction in pH. After 8 hours in a sweat booth, only 4% of the moisturizer had been removed from the skin. In addition, immersion testing proved outstanding results. At the end of the day, positive moisturization was felt, whereas most formulas fail in a few hours. The example products did not need to be altered for oily, normal, or dry skin, as the controlled release of the acids were found to balance and normalize all skin types, even the most sensitive skin.

8

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 Mauro, T. *Arch Dermatol. Res* 290(4): 215-222, 1998

What is claimed is:

1. A composition comprising an ammonium salt of a weak organic acid combined with a time release polymer base formulation wherein the composition releases ammonia when in skin contact to provide a gradual decrease in pH to 5.5 or less.
2. The composition of claim 1, wherein the pH of the composition gradually decreases to between 2.0 and 5.0 when in skin contact.
3. The composition of claim 2, wherein the pH of the composition gradually decreases to between 4.0 and 4.5 when in skin contact.
4. The composition of claim 1, wherein the weak organic acid is selected from salicylic acid, glycolic acid, lactic acid, malic acid, citric acid, and tartaric acid.
5. The composition of claim 1, wherein the weak organic acid is an alpha or beta hydroxy acid.
6. The composition of claim 5, wherein the beta hydroxy acid is salicylic acid.
7. The composition of claim 1, wherein the time release polymer base formulation is pharmaceutically acceptable.
8. The composition of claim 1, wherein said formulation is a solution, lotion or cream.
9. A method of enhancing skin sloughing, comprising formulating a selected organic weak acid with a pharmaceutically acceptable emulsifying agent, neutralizing the acid with ammonia to a pH of 6.5-6.9 to form a dispersed ammonium salt, applying said ammonium salt to skin wherein the pH gradually lowers to 5.5 or less as ammonia evaporates causing a gradual enhancement of skin sloughing.
10. The method of claim 9, wherein the pH of the composition gradually decreases to between 2.0 and 5.0 when in skin contact.
11. The method of claim 10, wherein the pH of the composition gradually decreases to between 4.0 and 4.5 when in skin contact.
12. The method of claim 9, wherein the emulsifying agent is a volatile emulsifying polymer that evaporates over a period of time to leave a water insoluble polymer on skin surface.
13. The method of claim 9, wherein the weak organic acid is selected from the group consisting of salicylic acid, glycolic acid, lactic acid, malic acid, citric acid and tartaric acid.
14. The method of claim 9, wherein the pH is lowered over a period of 3-4 hours.
15. The method of claim 9, wherein the weak organic acid is an alpha or beta hydroxy acid.
16. The method of claim 15, wherein the beta hydroxy acid is salicylic acid.
17. The method of claim 9, further comprising a polymer that is water soluble pH 6 to 7 wherein the polymer becomes insoluble at a pH 5 and below pH 5.

US 6,984,391 B2

9

18. The method of claim 17, wherein the water soluble polymer is ammonium carboxymethyl cellulose, ammonium alginate, ammonium carrageenate, ammonium polyacrylate, or ammonium VA/Acrylate.

19. A kit comprising the composition of claim 1, in suitable container form and directions for applying said composition for skin moisturization and sloughing.

20. The kit of claim 19, wherein the composition is ammonium salicylate in a skin protective polymer.

21. The kit of claim 20, wherein the skin protective polymer is water soluble at neutral pH and insoluble at pH 5 and below pH 5.

22. The kit of claim 20, wherein the skin protective polymer is ammonium carboxymethyl cellulose, ammonium alginate, ammonium carrageenate, ammonium polyacrylate, or ammonium VA/Acrylate.

23. A method for treating acne, comprising applying an ammonium salt of salicylic acid dispersed in a formulation with an emulsifying agent and a lipid with sufficient water to allow gradual release of ammonia when in skin contact wherein the pH of the skin surface gradually lowers to a pH of 5.5 or less.

24. The method of claim 23, wherein the skin surface pH gradually decreases to between 2.0 and 5.0 when the composition is in skin contact.

10

25. The method of claim 24, wherein the skin surface pH gradually decreases to between 4.0 and 4.5 when the composition is in skin contact.

26. The method of claim 23, wherein the emulsifying agent is combined with a polymer and wherein the emulsifying agent evaporates over a period of time to leave a water insoluble emollient and/or polymer on the skin surface.

27. A method of enhancing skin sloughing, comprising formulating a selected organic weak acid with a pharmaceutically acceptable emulsifying agent, neutralizing the acid with ammonia to a pH of 6.5-6.9 to form a dispersed ammonium salt, applying said ammonium salt to skin wherein the pH gradually lowers to 5.5 or less as ammonia evaporates causing a gradual enhancement of skin sloughing.

28. The method of claim 27, wherein the pH gradually decreases to between 2.0 and 5.0 when the ammonium salt is in skin contact.

29. The method of claim 28, wherein the pH gradually decreases to between 4.0 and 4.5 when the ammonium salt is in skin contact.

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(54) **PROCESS FOR PREPARING WATER
 SOLUBLE DITERPENES AND THEIR
 APPLICATIONS**

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(57) **ABSTRACT**

Aqueous solutions of diterpenes such as Forskolin, its congeners, analogs and derivatives, up to approximately 6% concentration, are prepared using suitably substituted cyclodextrin as a solubilizing agents. In the absence of cyclodextrin, some diterpenes such as Forskolin are soluble in water only to concentrations of about 0.001%. Such aqueous solutions find applications in topical and systemic use, as pharmaceutical, cosmeceutical, nutraceutical preparations containing diterpenes such as Forskolin and congeners.

7 Claims, No Drawings

US 6,960,300 B2

1

PROCESS FOR PREPARING WATER SOLUBLE DITERPENES AND THEIR APPLICATIONS

BACKGROUND OF INVENTION

1. Field of Invention

The invention describes methods to prepare clear solutions of diterpenes, such as Forskolol and its congeners that are sparingly soluble or insoluble in water, of concentrations 0.09% to 6%, for convenient use in ophthalmic preparations as well as in topical, oral, injectable and other dosage forms, for human and veterinary use.

2. Description of Prior Art

Certain active pharmaceutical ingredients are inherently insoluble or very sparingly soluble in water or in aqueous vehicles. Very often their intended use may require their application in water or in aqueous vehicles. To achieve therapeutically active concentrations of such water insoluble active pharmaceutical ingredients in stable form has always been actively pursued. While the technique of molecular structural manipulation of the active pharmaceutical ingredient that is insoluble in water could be adopted, incorporating structural features that promote aqueous solubility may result in the attenuation or modification of the intended desired pharmacological properties. Hence it may be most desirable to invent methods of solubilizing the active ingredients in their native structural form by other means.

Aqueous solubility of drugs is a desirable feature from many angles. Aqueous formulations are sterilizable by standard techniques such as filtration etc to render such preparations suitable for systemic administration. Also aqueous preparations are preferable in dermatological, gynecological, otological, rhinological and on mucous membrane applications. Especially useful are aqueous ophthalmic preparations of drugs.

Forskolin (CAS no 66575-29-9) is a naturally occurring labdane diterpene from *Coleus forskohlii* (Bhat, S. V.; Bajwa, B. S.; Dornauer, H.; de Souza, N. J.; Fehlabar, H.-W.; Tetrahedron Lett., (1977), 18, 1669). It has several desirable pharmacological properties.

Forskolin displays positive inotropic, antihypertensive and broncho-spasmolytic activity; (Bhat, S. V.; Dohadwalla, A. N.; Bajwa, B. S.; Dadkar, N.; Dornauer, H.; de Souza, N. J.; J Med Chem., (1983), 26, 486).

It lowers intraocular pressure (Caprioli J, Sears M.; Lancet (1983); April 30;1(8331):958-60; Badian M et al.; Klin Monatsbl Augenheilkd (1984);185:5226, Zeng S, et al. Yan Ke Xue Bao (1995);11:173-176, Lee P Y, et al.; Arch Ophthalmol (1987);105:249-252. Meyer B H, et al. S Afr Med J. (1987);71:570-571; Seto C, et al.; Jpn J Ophthalmol (1986);30:238-244.; Burstein N I et al. Exp Eye Res (1984);39:745-749; Brubaker R F et al. Arch Ophthalmol (1987); 105:637-641).

Diverse biological activities are observed by raising the levels of cAMP, and as a result activating protein kinase. Such properties have led to numerous uses of Forskolol. Due to such activities, more than 1500 citations dealing with the physiological properties of Forskolol appeared in Chemical Abstracts in 2001. However, Forskolol is highly insoluble in water.

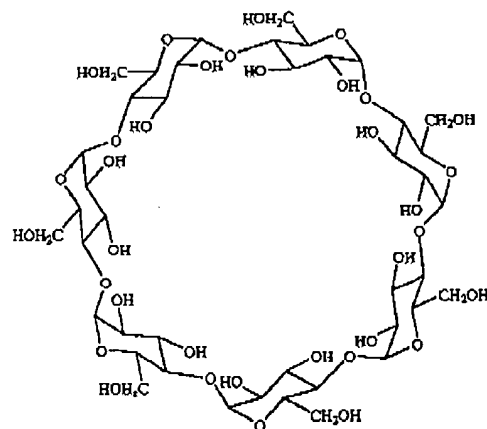
Intensive efforts have been made on the molecular manipulation of Forskolol to make such derivatives of Forskolol as will be water soluble. Such attempts have always met with mixed success (Lal, B.; Gangopadhyay, A.

2

K.; Rajagopalan, R.; Ghate, A. V.; Bioorganic & Medicinal Chemistry, (1998), 6(11), 2061-2073; Lal, B.; Gangopadhyay, A. K.; Gidwani, R. M.; Fernandez, M.; Rajagopalan, R.; Ghate, A. V.; Bioorganic & Medicinal Chemistry, (1998), 6(11), 2075-2083).

As an alternative to chemical manipulations of the drug molecular structure, physicochemical techniques of enhancing the solubility of the underivatized drug in water have been employed. Notable technologies include micellar solubilization using surface active ingredients, which will form water soluble micelles containing the drug. Another related technique is complexation of the drug molecule with a host molecule. The host molecule is usually one that has good solubility in water. The host molecule does not form any covalent bonds with the drug molecule but forms a weak complex through non-covalent interactions and the host molecule(s) keep the drug molecule(s) in water solution.

Cyclodextrins are cyclic oligosaccharides which have been recognized as useful pharmaceutical excipients. The common cyclodextrins are called α -, β -, γ - and δ -cyclodextrins depending on the number of glucose molecules in the cyclic oligosaccharide structure. These cyclodextrins are (α 1, 4)-linked oligosaccharides of α -D-glucopyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface. These molecules are not exactly perfect cylinders due to restriction of completely free rotation about their linking bonds of the units of the sugar molecule. They assume the shape of a torus or a truncated cone. The secondary hydroxyl groups line the wider edge of the rim while the primary hydroxyl groups line the narrow side of the torus. The solubilities of these molecules in water and the diameter of the central cavity have been known and published (Lofsson, T.; Brewster, M. E.; J Pharmaceutical Sciences, (1996), 85, 1017 & Rajewski, R. A.; Stella, V. J.; J Pharmaceutical Sciences, (1996), 85, 1142). The structure of β -cyclodextrin containing seven glucose units is shown as an example



Structure of Beta-cyclodextrin

The α -cyclodextrin has six anhydroglucose molecules in the ring; the γ - and δ -cyclodextrins have eight and nine respectively. The α -, β -, γ - and δ -cyclodextrins have their water solubilities at 25° C. (g/100 ml) 14.5, 1.85, 23.2 & 8.19 respectively. The α -, β -, γ - and δ -cyclodextrins are sometimes called natural cyclodextrins and their solubilities in water are at the lower end of the desirable range. Nevertheless they proved very good solubilizing agents for some of

US 6,960,300 B2

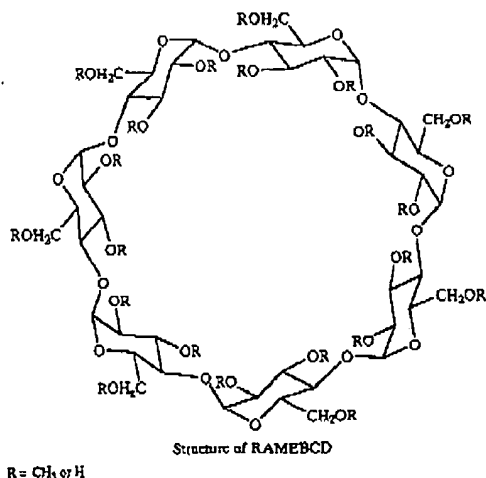
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the water insoluble molecules. To increase the aqueous solubilities of these natural cyclodextrins, molecular modifications of these α -, β -, γ - and δ -cyclodextrins have been carried out in the literature.

These modified cyclodextrins have much higher solubilities than their natural counterparts and they can be classified as Methylated derivatives of β -cyclodextrin, 2-hydroxypropylated β - and γ -cyclodextrins, sulfobutylated β -cyclodextrins, branched cyclodextrins, acylated β - and γ -cyclodextrins.

The cyclodextrins can be methylated by Kuhn-Trischmann methylation, Wacker's industrial method with methyl chloride under pressure and Hakamori methylation using methylhalogenide and sodium hydride (see, Szenté, L.; Szejtli, J.; Advanced Drug Delivery Reviews, (1996), 36, 17). The first two technologies have been used to produce randomly methylated cyclodextrin mixture. On the other hand Hakamori methylation is reported to produce a fully methylated heptakis 2,3,6-tri-O-methylated cyclodextrins. The introduction of methyl substituents in the place of the hydrogens of the hydroxy group of parent β -cyclodextrin dramatically improves the solubility of this randomly methylated cyclodextrin, referred in this invention as RAMEBCD versus the parent β -cyclodextrin.

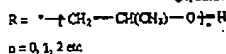
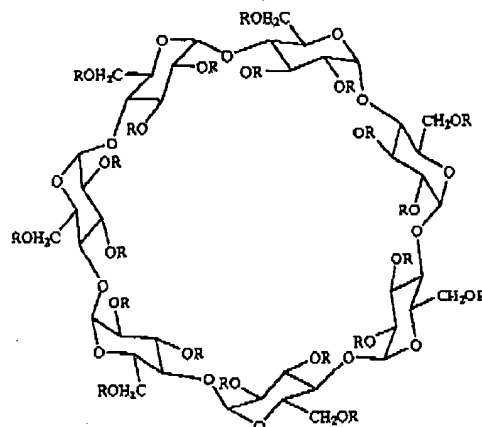
There are totally 21 hydroxyl groups (14 secondary hydroxyl groups and seven primary hydroxyl groups) in β -cyclodextrin. The aqueous solubility of RAMEBCD increases as the number of methyl groups reaches around 13-14 and decreases as methylation approaches 21 methoxy groups per molecule of β -cyclodextrin. An example of a commercially available RAMEBCD product can be cited the one produced by Wacker Chemie and marketed under the name CAVASOL® W7 M Pharma (CAS no 128446-36-6). Aqueous solubilities of such RAMEBCDs are typically ~220 g/100 ml of water. Such RAMEBCDs have an average degree of methylation ~1.7 to 1.9 per anhydroglucose unit. Such RAMEBCDs are available commercially and have very good aqueous solubilities as noted. The general structure of such RAMEBCDs are shown as follows



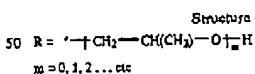
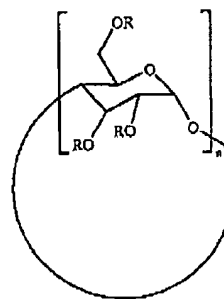
Reacting cyclodextrins with propylene oxide in alkaline solution results in substitution of the hydroxy groups in the cyclodextrins with 2-hydroxypropyl derivatives. A higher substitution of the hydroxyls with propylene oxide also

4

results in the formation of oligomeric hydroxypropylene oxide side chain formation. Such 2-hydroxy-propyl- β -cyclodextrin referred in this invention as HPBCD is represented by the following generic structure. Such materials are available commercially.



Similarly to HPBCD, γ -cyclodextrin can be hydroxypropylated to give hydroxypropyl γ -cyclodextrin, referred as HPGCD in this invention. Such materials are available commercially



A review on the applications of cyclodextrin in the ophthalmic field has appeared (Loftsson, T.; Jarvinen, T.; Advanced Drug Delivery Reviews, (1999), 36, 59). A patent, U.S. Pat. No. 6,346,273 describes the aqueous solubilization of forskolin through the use of polyvinylpyrrolidone and a surfactant, polyethyleneglycol-glycerol triisocaprate. The maximum solubility of Forskolin achieved in this patent is 0.2%.

U.S. Pat. No. 4,476,140 describes a composition and method for treatment of Glaucoma by administration of a therapeutically effective amount of a material selected from the group consisting of forskolin, colforsin and polyoxygenated Labdane derivatives. The active agent concentration of 0.1% to 4% is reported herein to be physiologically effective when administered as a topical suspension to the eye.

US 6,960,300 B2

5

U.S. Pat. Nos. 5,070,209, 4,978,678, 5,023,344, 4,871, 764 describe novel 12-halogenated forskolin derivatives, intermediates and processes for the preparation thereof, and methods for reducing intraocular pressure utilizing compounds or compositions.

EP0268256 describes novel 12-halogenated forskolin derivatives, intermediates and processes for their preparation, and methods for reducing intraocular pressure utilizing the compounds or compositions.

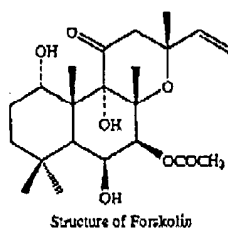
However these prior art references do not describe solubilization of unmodified forskolin to obtain clear aqueous solutions of concentrations of 1% or greater.

SUMMARY OF INVENTION

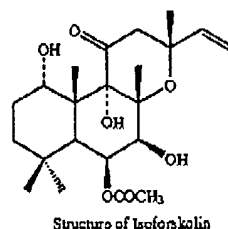
The invention describes the preparation of aqueous solutions of diterpenes such as Forskolin, that are sparingly soluble or insoluble in water, of concentrations up to approximately 6%. These solutions are prepared using suitably substituted cyclodextrin as a solubilizing agent. In the absence of cyclodextrin, Forskolin is almost insoluble in water yielding solutions of only about 0.001% concentration. Aqueous solutions of forskolin and/or its congeners, containing higher amounts of the active ingredient, can be used topically and systemically to provide diverse health benefits.

DETAILED DESCRIPTION

Forskolin has the following structure



A closely related isomer is called Isoforskolin and it has the following structure



Isoforskolin also has been reported to have many similar pharmacological properties as Forskolin. We have used these six commercially available cyclodextrins, namely, α -, β -, γ -cyclodextrins as well as their derivatized products such as RAMEBCD, HPBCD, HPGCD to solubilize the rather sparingly water soluble Forskolin.

To solubilize Forskolin using cyclodextrins, the chosen cyclodextrin and Forskolin are mixed in water in specific proportions. The aqueous solution is filtered to remove any undissolved particles to obtain a clear aqueous solution of Forskolin in water.

6

Alternatively, the cyclodextrin and Forskolin in certain proportions are dissolved in a suitable solvent such as ethanol or acetone or ethyl acetate. The solvent is removed to leave behind a white powder. Such powder freely dissolves in water as the examples will illustrate. Further, additives to the aqueous solution of Forskolin can also be added. These additives are usually used for maintaining sterility, pH maintenance, maintenance of osmolarity etc.

A wide variety of choice exists in the selection of such additives. While benzalkonium chloride is used in the illustrative example for preservative, one could equally choose from many others such as Benzethonium chloride, chlorobutanol, methyl paraben, propyl paraben, Thimerosal etc.

An antioxidant such as the disodium salt of EDTA is used to stabilize the preparation; other antioxidants such as sodium bisulfite, sodium metabisulfite, thiourea could be used also among others.

Especially for ophthalmic solutions, viscosity desired for an ophthalmic solution is in the range 25 and 50 cps. Viscosity enhancers such as polyvinyl alcohol, polyvinylpyrrolidone, methyl cellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose could be used.

The examples that are described below serve their purpose only as illustrative examples and do not limit in any way the broad scope of this invention.

ILLUSTRATIVE EXAMPLES

Example 1

Determination of the aqueous solubility of Forskolin. Forskolin (300 mg) was dried at 105° C. for 6 hours. Dried Forskolin 200 mg was stirred with 100 ml water for 48 hours for the determination of intrinsic solubility at ambient temperature. Resulting solution was filtered through 0.45 μ m nylon filter and analyzed for the content of Forskolin by HPLC. Content of Forskolin by HPLC 0.01 mg/ml or 0.001% w/v; In other words Forskolin has a solubility of ~0.001% w/v in water.

Example 2

Forskolin (98.5% assay, 25 mg) was added to 1 ml water containing in the dissolved state 500 mg Hydroxy propyl β cyclodextrin, HPBCD, (~50%). Suspension was agitated at 75 RPM in an isothermal shaker for 60 hours at temperature ~30° C. Resulting solution was filtered through 0.45 μ m nylon filter and analyzed for the content of Forskolin by HPLC 1.33 mg/ml or 0.133% w/v.

Example 3

Forskolin (98.5% assay, 50 mg) was added to 1 ml water containing 500 mg Hydroxy propyl γ -cyclodextrin in the dissolved state. (HPGCD) (~50%). Suspension was agitated at 75 RPM in an isothermal shaker for 60 hours at temperature ~30° C. Resulting solution was filtered through 0.45 μ m nylon filter and analyzed for the content of Forskolin by HPLC 1.52 mg/ml or 0.152% w/v.

Example 4

Experiments were performed by "changing" the crystallinity of Forskolin by recrystallizing from methylene chloride and from ethyl acetate. Resulting "amorphous" Forskolin was used for complexation with Hydroxypropyl γ -cyclodextrin HPGCD. Forskolin (29.3 mg) recrystallized with methylene dichloride (Forskolin assay 99.0%) was

US 6,960,300 B2

7

added to 3 ml water containing 1.5 gram Hydroxy propyl γ -cyclodextrin, HPGCD (~50%). Suspension was agitated at 75 RPM in an isothermal shaker for 160 hour at temperature 30° C. Resulting solution was filtered through 0.45 μ m nylon filter and analyzed for the content of Forskolin by HPLC 1.74 mg/ml or 0.174% w/v.

Example 5

Forskolin (30.3 mg) recrystallized with ethyl acetate (Forskolin assay 98.8%) was added to 3 ml water containing 1.5 gram Hydroxy propyl γ -cyclodextrin, HPGCD (~50%). Suspension was agitated at 75 RPM in an isothermal shaker for 160 hour at temperature 30° C. Resulting solution was filtered through 0.45 μ m nylon filter and analyzed for the content of Forskolin by HPLC 3.38 mg/ml or 0.338% w/v.

Example 6

Forskolin (98.5% assay, 330 mg) was added to 10 ml water containing 4 g of RAMEBCD (~40%). Suspension was agitated at 75 RPM in an isothermal shaker for 40 hours at temperature 30° C. Resulting solution was filtered through 0.45 μ m nylon filter and analyzed for the content of Forskolin by HPLC 20.46 mg/ml or 2.046% w/v.

Example 7

Solubility of Forskolin in water was determined at the different concentrations of RAMEBCD ranging from 5 to 66%. The relationship is nearly linear and indicates that the solubility of Forskolin is increased by increasing the concentration of RAMEBCD.

S.N.	% Concentration of RAMEBCD	% Forskolin w/v
1	5% RAMEBCD	0.09
2	10% RAMEBCD	0.272
3	15% RAMEBCD	0.767
4	20% RAMEBCD	1.15
5	40% RAMEBCD	2.746
6	53.28% RAMEBCD	4.165
7	66.6% RAMEBCD	5.029

Example 8

A typical aqueous formulation of Forskolin with a cyclodextrin is prepared as follows, RAMEBCD, being used as the example of cyclodextrin RAMEBCD (100 g) is taken in a one liter flask with mechanical or magnetic stirring facility. Forskolin (5.5 g) was charged into the flask. Water (400 ml) is charged to the flask and the contents were agitated at room temperature. A clear solution is obtained. If any undissolved Forskolin particles are seen, they are resuspended and stirred. Benzalkonium chloride (50 mg) and Disodium EDTA (500 mg) are added and dissolved in the flask. The pH of the contents could be adjusted to the desired range with the help of 0.1N sodium hydroxide. (usually pH range 3.5 to 7.5). Calculated amount of sodium chloride solution is added to maintain the osmolarity of the solution equivalent to that of 0.9% sodium chloride. The total volume of the solution is made up to 500 ml after sterile filtration. A solution thus prepared has approximately 1% of Forskolin in the dissolved state. Other cyclodextrins also could be used and depending on the cyclodextrin used, the dissolved content of Forskolin in water differed.

Example 9

Forskolin (50 mg) was dissolved in 5 ml acetone, and 1 gram of RAMEBCD was dissolved in 5 ml acetone sepa-

8

ately. Both the solutions were mixed together and solvent acetone was evaporated under reduced pressure. Residue was dried and dissolved in 5 ml water. This residue dissolved very easily within 1 hour of stirring forming a clear colorless solution.

Example 10

Isoforskolin also could be used in place of Forskolin. In one preparation, Isoforskolin (50 mg) was suspended in water containing a suitable amount of a cyclodextrin, for example, R AMEB CD (20 g) in about 100 ml water. After agitation at room temperature, the solution was filtered and the resulting solution was analyzed by HPLC which showed the presence of Isoforskolin approximately 0.5%; The amount of dissolved Isoforskolin could be altered by changing the amount of RAMEBCD.

Example 11

An illustrative example of the biological activity of the preparation is presented. The anti-glaucoma activity of the forskolin composition was studied in albino rabbits. A 1% solution of Forskolin in water as described in example 8 was used for the experiments

Study design: Animal model: Albino rabbit Number of groups: 4 Number of animals in each group: 6 in treatment group and 2 in control group

Materials and methods: Six albino rabbits of New Zealand strain, of both sexes, weighing 1.0-1.5 lb were chosen. The rabbits were housed in clean and well-ventilated open space. Each rabbit was fed with standard diet daily and water was administered ad libitum throughout the study.

Ocular hypertension was induced by the method reported by Bonomi et al (Invest Ophthalmol. 1976 September; 15(9):781-4.) The rabbits were given 0.3 ml subconjunctival injection of Betnesol containing betamethasone sodium 4 mg/ml, every day to each eye for a period of three weeks (the intraocular pressure (IOP) at third week was maximum as per literature). Local anesthetic propracaine eye drops were used prior to subconjunctival injections.

In each rabbit the left eye was kept as control for glaucoma and the right eye was treated for glaucoma using Forskolin, Timolol, and the placebo.

For each treatment, the IOP readings were measured at intervals of 30 minutes up to 210 minutes using the non-contact tonometer (NCT).

Results

Time (mins)	IOP Readings* (mmHg)		
	Placebo	Timolol Right eye (treated)	Forskolin
0	14	13	14
30	13	8	7
60	12	7	6.5
90	11.5	5	4.5
120	11	4	5
150	10.5	3.5	6
180	10	4.5	7
210	9	6	7.5

*Average of 6 determinations

IOP of left eye (control) ranged between 12-13 mmHg.

IOP of control group animals ranged between 4-4.5 mmHg.

Statistical Analysis: The IOP readings of the placebo, Forskolin and Timolol were subjected to ANOVA (one way).

US 6,960,300 B2

9

The p value was 0.0022 which is very significant, indicating that the variation in column means is not by chance.

The IOP readings of the placebo and forskolin were subjected to "t" test to determine whether the medians of Forskolin and the placebo differ significantly. The p value was found to be 0.0177 which is considered significant. Similarly, the IOP readings of the placebo and Timolol had a "p" value of 0.0087, which is again significant.

The IOP readings of Forskolin and Timolol were also subjected to "t" test. The p value was found to be 0.3999, which is not considered significant, implying that the activity of forskolin preparation is not significantly different from Timolol.

Conclusion: The Forskolin composition has anti-glaucoma activity comparable to Timolol.

What is claimed is:

1. A method of solubilizing at least one natural or synthetic forskolin, isoforskolin, or 7-deacetylforskolin in water, the method comprising:

1) suspending forskolin, isoforskolin or 7-deacetylforskolin in water containing a complexing/solubilizing cyclodextrin agent;

2) agitating at room temperature; and

3) filtering to obtain a clear aqueous solution containing 0.09% to 6% of forskolin, isoforskolin, or 7-deacetylforskolin.

2. The method as claimed in claim 1, wherein the at least one natural forskolin, isoforskolin, or 7-deacetylforskolin is obtained from *Coleus forskohlii*.

3. The method as claimed in claim 1, wherein the complexing/solubilizing cyclodextrin agent is selected from the group consisting of α -, β -, γ -cyclodextrins or their derivatized products, randomly methylated β -cyclodextrin (RAMEBCD), 2-hydroxy-propyl- β -cyclodextrin (HPBCD), and hydroxypropyl γ -cyclodextrin (HPGCD).

4. The method as claimed in claim 3, wherein prior to step 1), the cyclodextrin agent and forskolin, isoforskolin, or 7-deacetylforskolin are dissolved in a solvent under

10

agitation, wherein the solvent is selected from the group consisting of ethanol, acetone, ethyl acetate, and methylene chloride, followed by removal of the solvent and suspending and dissolving the residue in water.

5. The method of claim 1, wherein the clear aqueous solution is suitable for ophthalmic, topical and systemic uses.

6. A method of solubilizing at least one natural or synthetic forskolin, isoforskolin, or 7-deacetylforskolin in water, the method comprising:

1) dissolving forskolin, isoforskolin or 7-deacetylforskolin in an organic solvent selected from ethanol, acetone, ethyl acetate and methylene chloride;

2) recrystallizing forskolin, isoforskolin or 7-deacetylforskolin from the organic solvent;

3) complexing the forskolin, isoforskolin or 7-deacetylforskolin in water containing a complexing/solubilizing cyclodextrin agent selected from the group consisting of α -, β -, γ -cyclodextrins or their derivatized products, randomly methylated β -cyclodextrin (RAMEBCD), 2-hydroxy-propyl- β -cyclodextrin (HPBCD), and hydroxypropyl γ -cyclodextrin (HPGCD);

4) agitating at room temperature for 4 to 160 hours; and

5) filtering to obtain a clear aqueous solution containing 0.09% to 6% of forskolin, isoforskolin, or 7-deacetylforskolin.

7. A method of solubilizing at least one natural or synthetic forskolin, isoforskolin, or 7-deacetylforskolin in water, the method comprising: 1) suspending forskolin, isoforskolin or 7-deacetylforskolin in water containing randomly methylated β -cyclodextrin (RAMEBCD);

2) agitating at room temperature; and

3) filtering to obtain a clear aqueous solution containing 0.09% to 6% of forskolin, isoforskolin, or 7-deacetylforskolin.

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